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Allozyme and mitochondrial DNA variability within the New Zealand damselfly genera *Xanthocnemis*, *Austrolestes*, and *Ischnura* (Odonata)

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Abstract We collected larval damselflies from 17 sites in the North, South and Chatham Islands, and tested the hypotheses that: (1) genetic markers (e.g., allozymes, mtDNA) would successfully discriminate taxa; and (2) the dispersal capabilities of adult damselflies would limit differentiation among locations. Four species from three genera were identified based on available taxonomic keys. Using 11 allozyme loci and the mitochondrial cytochrome *c*-oxidase subunit I (COI) gene, we confirmed that all taxa were clearly discernible. We found evidence for low to moderate differentiation among locations based on allozyme (mean $F_{ST} = 0.09$) and sequence (COI) divergence (<0.034). No obvious patterns with respect to geographic location were detected, although slight differences were found between New Zealand's main islands (North Island, South Island) and the Chatham Islands for *A. colensonis* (sequence divergence 0.030–0.034). We also found limited intraspecific genetic variability based on allozyme data ($H_{exp} < 0.06$ in all cases). We conclude that levels of gene flow/dispersal on the main islands may have been sufficient to maintain the observed homogeneous population structure, and that genetic techniques, particularly the COI gene locus, will be a useful aid in future identifications.

Keywords allozymes; COI; dispersal; DNA barcoding; gene flow; population genetics; Zygoptera

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INTRODUCTION

Molecular genetic techniques such as enzyme electrophoresis and, particularly, DNA sequencing have become increasingly accessible. They are used in a variety of ecological/evolutionary applications, including studies of population genetics, phylogeny and phylogeography (Avise 2004). Molecular approaches have aided species identification (Tautz et al. 2003; Hebert et al. 2003) in a range of aquatic insects in North America (e.g., Sweeney et al. 1987; Jackson & Resh 1998), Europe (e.g., Wilcock et al. 2001) and Australasia (e.g., Hughes et al. 1998; Smith & Collier 2001; Hogg et al. 2002).

Comparatively few studies have focused on odonates (e.g., Geenen et al. 2000), even though many

species of Odonata are now classed as endangered or declining in range (Freeland & Conrad 2002; Watts et al. 2004). Molecular studies on odonates are needed to test hypotheses on population structure and variability relative to taxon, gene flow/dispersal capabilities and adult longevity (e.g., Bilton et al. 2001).

The potential for dispersal/gene flow among odonate populations in discrete habitats could be high, because adult dragonflies and damselflies may live for more than 30 days actively hunting for food and mating, and some are known to make annual migrations (Freeland et al. 2003). Their presence on distant offshore islands of relatively recent geological origin (e.g., Chatham Islands; Rowe 2000), predicts minimal genetic structure among populations. On the other hand, other Australasian taxa show strong genetic structuring among discrete aquatic systems (Smith & Collier 2001; Hogg et al. 2002; Smith et al. 2006), and even among reaches of the same river system (Hughes et al. 1998). For example, Smith & Collier (2001) and Hogg et al. (2002), found a north-south pattern of differentiation in New Zealand for *Orthopsyche fimbriata* (Trichoptera) and *Archicauliodes diversus* (Megaloptera), respectively, increasing with geographic distance. Similar patterns have been observed in North America (Sweeney et al. 1987). In some cases, levels of genetic differentiation have been strong enough to suggest the existence of previously undescribed and/or cryptic species (Sweeney & Funk 1991; Jackson & Resh 1998).

The potential underestimation of biodiversity has serious implications for conservation particularly of odonates (e.g., incidental loss of range-restricted/rare species following uncontrolled habitat destruction). We used available biogeographic and natural history data to test the hypotheses that: (1) molecular genetic techniques (allozyme electrophoresis, mtDNA sequencing) would successfully discriminate the New Zealand damselfly taxa; and (2) established species would show limited levels of genetic differentiation among habitats.

METHODS

Study taxa

Six species of damselflies in three genera are recognised in New Zealand. (1) *Austrolestes colensonis* (White, 1846) is widespread on all main islands. (2) *Ischnura aurora* (Brauer, 1865), a recent arrival, is found only on the North Island (Rowe 2000). (3) Of four species of *Xanthocnemis*, only *X. zealandica* (McLachlan, 1873) is widespread, and is probably the most common odonate species throughout the country (Rowe 1987). The other three are much more restricted. For example, *X. sobrinia* (McLachlan, 1873) is known only from the northern North Island; *X. sinclairi* (Rowe, 1987) from alpine tarns in the headwaters of a single river (Rowe 2000); and *X. tuanuii* (Rowe, 1981) from Chatham Islands.

Study sites and collection of animals

We sampled several appropriate aquatic habitats throughout New Zealand (including Chatham Islands) between 1998 and 2005. We collected larval damselflies from a total of 17 sites (Fig. 1; Table 1), using a sweep net in submerged aquatic macrophytes or overhanging bank-side vegetation. We attempted to collect up to 40 individuals per species for the allozyme analyses, but their relatively low densities and patchy distribution usually made this impossible. *Austrolestes* and *Ischnura* were recovered in very low numbers (<5 per site in all cases). All generic and/or species designations were morphologically confirmed from Rowe (2000). The species distribution and sites of origin of all specimens used for the allozyme and mitochondrial DNA (mtDNA) analyses are shown in Fig. 1, Table 1.

Allozyme analyses

Heads of individual damselflies were removed and homogenised in 15 µl of buffer from a stock solution consisting of: distilled water (100 ml), NADP (10 mg), β-mercaptoethanol (100 µl), and detergent Tween 80 (100 µl) (Richardson et al. 1986). We used cellulose acetate gel electrophoresis (Helena Super Z-12 applicator kit; 76 × 76 mm Titan III cellulose acetate plates) and staining recipes outlined in Hebert & Beaton (1993). Running conditions consisted of 15–20 min at 200 mV. Animals were screened for allozyme activity for several enzyme systems and following this preliminary screening, we selected the following nine enzyme systems for analyses: isocitrate dehydrogenase (IDH: EC 1.1.1.42), lactate dehydrogenase (LDH: EC 1.1.1.27), 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44), Glycerol-3-phosphate dehydrogenase (GPDH: EC 1.1.1.8), peptidase (PEP: EC 3.4.11/13), phosphoglucomutase (PGM: EC 2.7.5.1), malate dehydrogenase (MDH: EC 1.1.1.37), phosphoglucose isomerase (PGI: 5.3.1.9). Three enzymes (IDH, PEP, MDH) were scorable at two loci each providing a total of 11 presumptive loci. However,

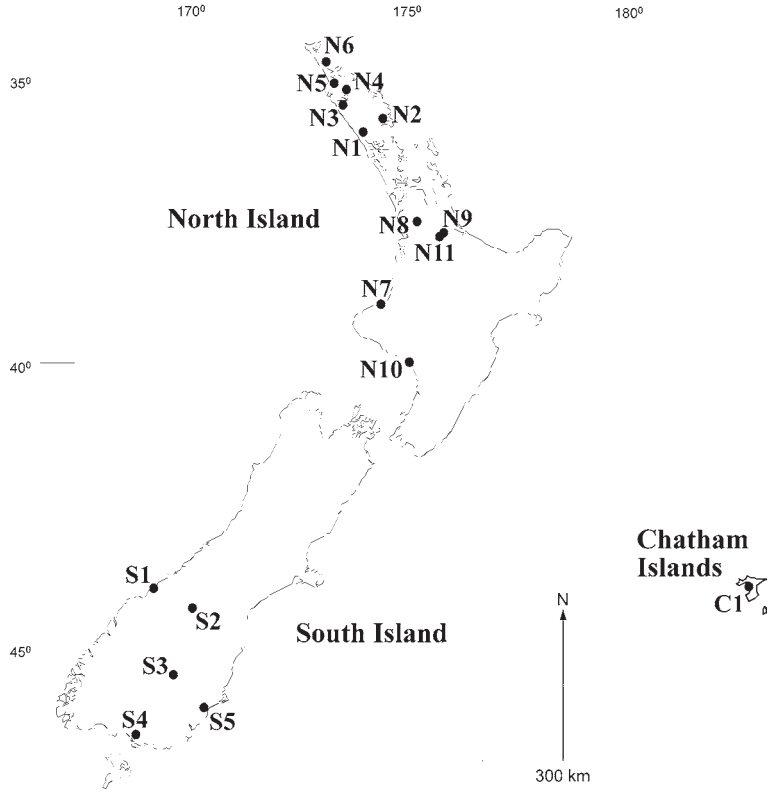

Fig. 1 Study area, showing collection sites for damselflies on the North Island, South Island and Chatham Island of New Zealand. Further details for each site are provided in Table 1.

Table 1 Sampling sites for damselflies *Xanthocnemis* spp. (X), *Austrolestes colensonis* (A), and *Ischnura aurora* (I). Site codes refer to Fig. 1. C = Chatham Island; N = North Island; S = South Island.

Site	Name	Date	Lat. (S)	Long.	Habitat	Species	Allozyme	mtDNA
C1	Point Gap	25 Feb 2000	44°04′	176°23′W	2nd order stream	X,A	Xx11 Ax8	Xx3 Ax1
N1	Dargaville	16 Feb 2000	35°55′	173°54′E	2nd order farm stream	X,A	Xx13 Ax4	Xx2
N2	Whangerei	18 Feb 2000	35°43	174°19′E	4th order stream	X,I	Xx22 Ix1	Xx2
N3	Whirinaki Armco culvert	17 Feb 2000	35°28′	173°28′E	2nd order stream	X,I	Xx6 Ix3	Xx2 Ix1
N4	Mangatina Scenic reserve	17 Feb 2000	35°12′	173°30′E	Spring-fed 1st order stream	X	Xx12	Xx1
N5	near Kaitia	17 Feb 2000	35°07′	173°17′E	2nd order stream	X	Xx17	Xx1
N6	Houhora	17 Feb 2000	34°48′	173°07′E	2nd order stream	X,I	Xx4 Ix1	Xx1
N7	Waiwhakaiho River	19 Sep 1999	39°02′	174°08′E	2nd order stream	X	Xx3	
N8	Lake Waahi	11 Aug 1998	37°34′	175°08′E	Small lake	X,I	Xx5 Ix4	Xx3
N9	Waitoa River	12 Aug 1998	37°31′	175°38′E	2nd order stream	X,I	Xx20 Ix2	Xx5 Ix1
N10	Whanganui River	18 Nov 2001	39°55′	175°01′E	3rd order stream	A		Ax1
N11	Piako River	9 May 2005	37°39′	175°32′E	River	X		Xx3
S1	Heritage Park Lodge Pond	18 Jan 1998	43°52′	169°01′E	Pond	X	Xx9	Xx1
S2	Lake Ruatanewha (Twizel)	18 Jan 1998	44°17′	170°04′E	Lake	X,A	Xx17 Ax1	
S3	Butchers Dam	16 Jan 1998	45°18′	169°20′E	Small lake	X	Xx12	Xx2
S4	Waihopai River	1 Apr 2001	46°39	168°38′E	River	A		Ax2
S5	Lee Creek	12 Dec 1998	45°55	170°10′E	Creek	X		Xx2

because allozyme analyses are sensitive to enzyme degradation and/or loss of signal, it was not always possible to score all enzymes/loci for a particular individual. Accordingly, in some instances, sample sizes varied slightly among loci.

Alleles were scored in order of increasing mobility; those migrating closest to the anode were designated "A", and sequentially slower alleles "B", "C" etc. For enzymes with more than one locus (e.g., IDH), these were assigned in order of increasing electrophoretic mobility such that *IDH-2* was closer to the anode than *IDH-1*. In order to verify the accuracy of allelic designations, gel line-ups (*sensu* Richardson et al. 1986), were used, and at least one individual from a previous run was included on subsequent gels.

We used BIOSYS-1 (Swofford & Selander 1981) to calculate: (1) allele frequencies; (2) measures of genetic variability (mean number of alleles per locus, percentage of polymorphic loci where the frequency of the most common allele was <95%, expected and observed heterozygosity); (3) agreement of genotype frequencies with predictions of Hardy-Weinberg equilibrium; (4) Wright's (1978) F_{IS}, and F_{ST}; and (5) Nei's (1978) unbiased genetic distance. *F*-statistics for each locus were tested for significance using the formulae given by Waples (1987).

mtDNA analyses

DNA was extracted from whole individuals (1–5 individuals per site) using the DNeasy Tissue kit (Qiagen Inc., Hilden, Germany) as per the manufacturer's instructions, except that we incubated the sample at 56°C for 24 h and used 60 µl to elute the DNA. PCR amplification was carried out using a 50 µl reaction volume consisting of 2 µl of DNA, 1 × PCR buffer (Roche, Penzberg, Germany), 2.2 m*M* $MgCl_2$, 0.2 m*M* of each dNTP (Boehringer Mannheim, Mannheim, Germany), 1.0 µ*M* of each primer, and 1.0 unit of *Taq* DNA polymerase (Roche) on a Eppendorf Mastercycler gradient thermocycler. A 680 base pair fragment of the mtDNA gene cytochrome *c*-oxidase I (COI) gene was amplified using the primers CO1-2F (5′-TYG AYCCIDYIGGRGGAGGAGATCC-3′) and CO1-2R (5′-GGRTARTCWGARTAWCGNCGWGGTA T-3′) (Otto & Wilson 2001). The thermal cycling conditions were: initial denaturation at 94°C for 60 s; followed by 40 cycles of denaturation at 94°C for 20 s; annealing at 50°C for 30 s; and extension at 72°C for 90 s; with a final extension at 72°C for 5 min. Sequencing was performed using the same primers as those used for PCR amplification on an ABI 377XL automated sequencer (Applied Biosystems Inc., Foster City, CA) or a MegaBACE DNA Analysis System (Amersham Biosciences, Buckinghamshire, England) at the University of Waikato DNA sequencing facility.

Sequences were aligned using SEQUENCHER (Gene Codes version 4.1.2) and verified as being derived from insect DNA using GenBank BLASTn searches. We used χ^2-tests, as implemented in PAUP* 4.0b10 (Swofford 2002), to determine whether the assumption of equal base frequencies among sequences was violated on: (1) all sites; (2) parsimony-informative sites only; and (3) with the third codon position only. We then constructed phylogenetic trees using PAUP* 4.0b10. A maximum likelihood (ML) phylogram was constructed using the GTR+Γ model (–1nL (hLRTs) = 2390.89: rate matrix = 1.6197, 6.0854, 3.1696, 0.8274, 14.4680; Γ = 0.2286; with base frequencies A = 0.3273, C = 0.1606, G = 0.1596, T = 0.3524) (selected using Modeltest 3.7; Posada & Crandel 1998).

We also implemented a maximum parsimony (MP) analysis using the branch and bound search option with unweighted characters. One dragonfly species (*Aeshna brevistyla*) was sequenced for use as an outgroup taxon (accession number: EU219906), in addition to *Orthetrum triangulare melania* obtained from GenBank (accession number: AB126005).

We compared the ML and MP trees in order to minimise the potential for error that may arise from assumptions inherent in phylogenetic reconstruction, and by bootstrap analysis with 100 pseudoreplicates (identical sequences were removed to decrease analysis time) for the ML tree (Felsenstein 1985). A Templeton (Wilcoxon signed-ranks) test was used to determine if significant differences existed between the trees. All sequences have been submitted to GenBank (accession numbers: EU219872–EU219905).

RESULTS

Allozymes

All three genera were clearly discernible on the basis of allozyme data with fixed differences observed at several loci (Table 2), or, in the case of *I. aurora* versus *X. zealandica*, at all loci.

Two species of *Xanthocnemis* were found on Chatham Islands; one having the same allelic composition to *X. zealandica* found elsewhere in New Zealand, and a single individual (presumably

Table 2 Allele frequencies at 11 allozyme loci for damselflies (Odonata) collected from 14 sites in New Zealand. Site codes (for *Xanthocnemis zealandica*) refer to Fig. 1. A = *Austrolestes*, I = *Ischnura*, Xt = *X. tuanuii*. Sample sizes (*N*) are shown immediately below header row.

	CI	N1	N2	N3	N4	N5	N6	N7	N8	N9	S1	S2	S3	A	I	Xt
N	11	13	22	6	12	17	4	3	5	20	9	17	12	13	11	1
IDH-1																
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
B														1.00	1.00	1.00
IDH-2																
A															1.00	
B												0.03				
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00		
D																1.00
LDH																
A															1.00	
B			0.16	0.17	0.21	0.03		0.17		0.08						
C	1.00	1.00	0.84	0.83	0.79	0.97	1.00	0.83	1.00	0.92	1.00	1.00	1.00			1.00
D														1.00		
6PGDH																
A															1.00	
B														1.00		
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00
GPDH																
A														1.00		
B															1.00	
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00
PEP-1																
A								0.17								1.00
B	0.95	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00		
C	0.05															
D															1.00	
PEP-2																
A															1.00	
B														1.00		1.00
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
PGM																
A														1.00		
B															1.00	
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00
MDH-1																
A															1.00	
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
C																1.00
MDH-2																
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.78	1.00	1.00			
B											0.22				1.00	
C										0.03				1.00		1.00
GPI																
A		0.04	0.02												1.00	
B										0.03						
C	0.94	0.96	0.98	1.00	1.00	1.00	0.87	1.00	1.00	0.97	0.94	0.97	1.00			
D														1.00		
E	0.06						0.13				0.06	0.03				
F																1.00

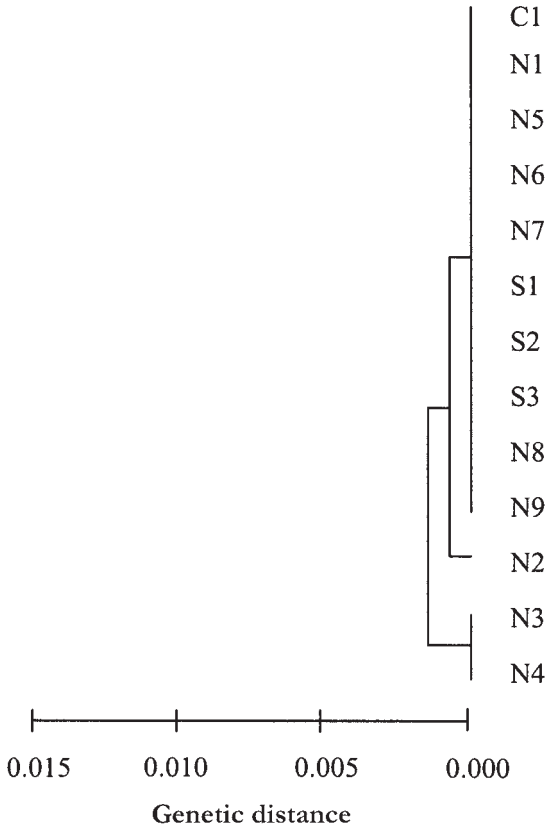

Fig. 2 Unweighted paired group method of analysis (PGMA) dendrogram for *Xanthocnemis zealandica* on the North Island and South Island of New Zealand based on an analysis of 11 allozyme loci using Nei's (1978) genetic distance. Site codes refer to Fig. 1.

X. tuanuii), showing fixed differences at 10 of 11 loci (Table 2). Of the 11 loci assessed, both *A. colensonis* and *I. aurora* showed no within-species allelic variability among sites (and hence appear as single entries in Tables 2, 3). *Xanthocnemis zealandica* also had limited allelic variability, with only five of 11 loci showing more than one allele among all sites. Individuals from the South Island and Chatham Island sites showed variability at only two of 11 loci (Table 2).

Measures of genetic variability were similarly limited (Table 3). The mean number of alleles per locus was <1.3 in all cases, and observed/expected heterozygosity was low (<0.06 in all cases, and usually <0.03) (Table 3). The percentage of polymorphic loci (95% criterion) for *X. zealandica* was also low (0–18%). Frequencies of genotypes for all variable loci were in agreement with Hardy-Weinberg expectations (exact probability method).

F_{IS} values were generally negative, with the exception of *LDH*. However, none of these values was significant (Table 4). Differentiation among populations of *X. zealandica* was low to moderate (mean F_{ST} = 0.09; Table 4). Nei's (1972) distance values were <0.01 in all cases, suggesting minimal allelic differences among sites. No obvious pattern was found with respect to geographic location or habitat type (e.g., lotic versus lentic habitats) (Fig. 2).

mtDNA

A 598 base pair alignment of the mitochondrial COI gene was used in all analyses for the 34 damselflies of all four species (three genera) from 15 sites and

Table 3 Measures of genetic variability (+SE) based on 11 allozyme loci for *Xanthocnemis zealandica* collected from 13 sites throughout New Zealand. Site designations refer to Table 1.

Population	Mean sample size/ locus	Mean no. of alleles/locus	% polymorphic loci	H_{obs}	H_{exp}
C1	10.4 (0.4)	1.2 (0.1)	9	0.02 (0.01)	0.02 (0.01)
N1	11.9 (1.0)	1.1 (0.1)	0	0.01 (0.01)	0.01 (0.01)
N2	18.5 (1.2)	1.2 (0.1)	9	0.03 (0.02)	0.03 (0.03)
N3	6.0 (0.4)	1.1 (0.1)	9	0.03 (0.03)	0.03 (0.03)
N4	9.6 (0.9)	1.1 (0.1)	9	0.04 (0.04)	0.03 (0.04)
N5	15.4 (1.0)	1.1 (0.1)	0	0.01 (0.01)	0.01 (0.01)
N6	3.7 (0.1)	1.1 (0.1)	9	0.02 (0.02)	0.02 (0.02)
N7	2.9 (0.1)	1.2 (0.2)	18	0.06 (0.04)	0.06 (0.04)
N8	5.0 (0.0)	1.0 (0.0)	0	0.00 (0.00)	0.00 (0.00)
N9	19.3 (0.3)	1.3 (0.1)	9	0.02 (0.01)	0.02 (0.01)
S1	6.8 (0.2)	1.1 (0.1)	9	0.01 (0.01)	0.01 (0.01)
S2	16.1 (0.7)	1.2 (0.1)	0	0.01(0.01)	0.01 (0.01)
S3	11.2 (0.4)	1.0 (0.0)	0	0.00 (0.00)	0.00 (0.00)

the two additional genera included as outgroup taxa. No insertions, deletions or stop codons were detected. There were 164 parsimony-informative sites with an additional 36 variable sites. The nucleotide composition of all sequences was: A = 31%, T = 33%, C = 17% and G = 18%. The assumption of homogeneity of base frequencies was supported using all sites ($\chi^2_{105} = 26.72$, $P = 1.00$), parsimony-informative sites ($\chi^2_{105} = 94.40$, $P = 0.76$), and for third codon sites ($\chi^2_{105} = 70.22$, $P = 1.00$). There was 14% sequence divergence (uncorrected *P* values) between the two outgroup taxa, and 17–20% divergence was found between these and the ingroup. Within the ingroup, 21 different haplotypes were found with pairwise sequence divergences ranging from 0 to 18% (Table 5).

Divergence values (uncorrected) ranged from 0 to 1.6% among individuals of *X. zealandica* to greater than 15% between *Xanthocnemis* spp. and *Austrolestes* (Table 5). Divergences between *X. zealandica* and a second *Xanthocnemis* species found in Chatham Islands (presumably *X. tuanuii*) was 7%. A Templeton (Wilcoxon signed-ranks) test examining tree similarity showed there were no significant differences between the ML and one (chosen by closest similarity to the consensus tree) of the 345 most parsimonious trees ($P < 0.05$). Here, we present the ML tree (Fig. 3), which clearly showed the three genera forming separate groups, with *X. tuanuii* forming a sister group with *X. zealandica*.

DISCUSSION

All taxa were readily discernible on the basis of both allozyme and mtDNA data. mtDNA (COI) divergence values within and among taxa were comparable with those from other arthropod taxa. In particular, minimum divergence values between species of *Xanthocnemis* (6–7%) were similar to divergence values found among species of Lepidoptera and Collembola (Hebert et al. 2003; Hogg & Hebert 2004).

The COI data showed limited sequence divergence among individuals of *I. aurora* (0.002) and *X. zealandica* (0–0.012). Given the small size and young age of Chatham Islands, the mitochondrial differences among *X. tuanuii* populations were expected to be small. In contrast, individuals of *Austrolestes* were found to have higher levels of sequence divergence (0.007–0.034), and divergences between the individuals from Chatham Island and the three mainland New Zealand populations (c. 3%) approached levels consistent with separate species in other insect taxa (e.g., lepidopterans; Hebert et al. 2003). Accordingly, a more detailed examination of the specific status of mainland and Chatham Island *Austrolestes* populations is warranted.

Levels of allozyme variability were low within all species, particularly in *A. colensonis* and *I. aurora*. One possible explanation is that our samples of these species were too small to detect any allelic variation ($n = 11$ and 10, respectively). In the case of the recently-arrived *I. aurora*, minimal variation is consistent with a founder effect (Rowe 1987). However, the absence of variation for *A. colensonis* even among widely separated sites (e.g., Chatham Islands versus North and South Islands), was surprising given that at least some differences were expected based from previous work (e.g., Trewick 2000; Hogg et al. 2002; Stevens & Hogg 2004; McGaughran et al. 2006). Accordingly, these available data suggest

Table 4 Wright's (1978) F_{IS} and F_{ST} values based on 11 allozyme loci for *Xanthocnemis zealandica* collected from 13 sites in New Zealand. Site designations refer to Table 1. Only significant deviations from zero are shown, all others were not significantly different to zero ($P > 0.05$).

Locus	F_{IS}	F_{ST}
IDH-2	–0.03	0.027
LDH	0.13	0.107 ($P < 0.01$)
PEP-1	–0.16	0.129 ($P < 0.001$)
MDH-2	0.03	0.02
GPI	–0.08	0.05
Mean	–0.12	0.09 ($P < 0.05$)

Table 5 Average pairwise divergence values (uncorrected *P* values) for mitochondrial haplotypes between *Austrolestes colensonis*, *Ischnura aurora*, *Xanthocnemis tuanuii*, and *X. zealandica.*

	A. colensonis	*I. aurora*	*X. tuanuii*	*X. zealandica*
A. colensonis	0.007–0.034			
I. aurora	0.180–0.185	0.002		
X. tuanuii	0.176–0.184	0.140–0.142	–	
X. zealandica	0.160–0.180	0.151–0.158	0.063–0.074	0–0.012

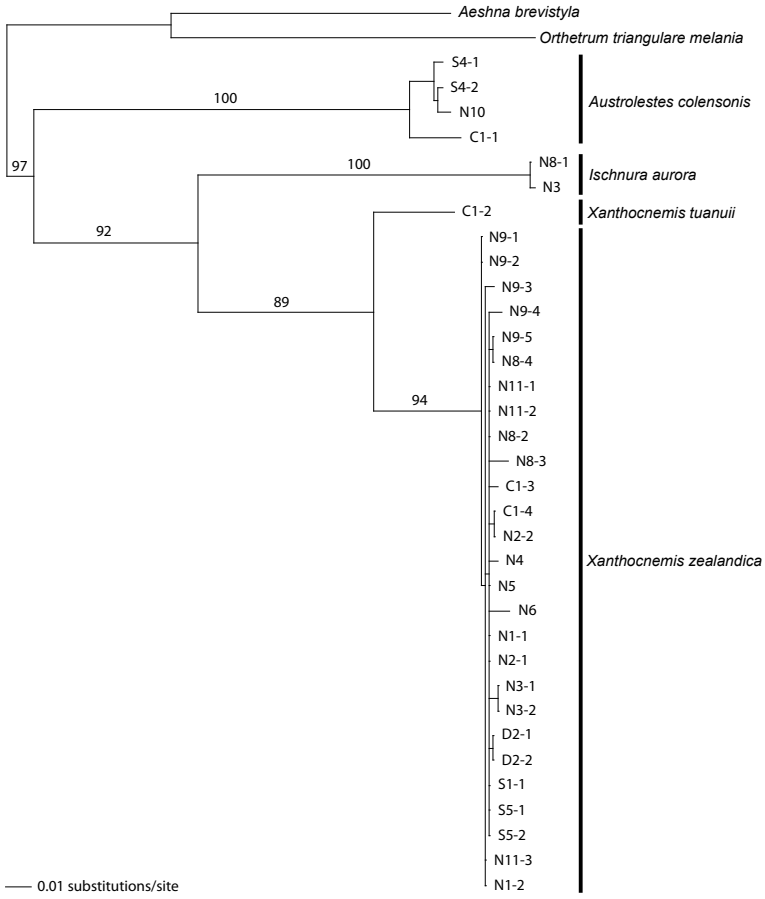

Fig. 3 Maximum likelihood (ML) tree for the three New Zealand damselfly genera: *Xanthocnemis*, *Ischnura* and *Austrolestes* based on a 598 bp fragment of the mitochondrial DNA (COI) gene with bootstrap values (100 replicates) shown at the nodes. Site codes refer to Fig. 1. Numbers in brackets following sites N9, N11, N3, S3, and S5 indicate number of individuals with identical haplotypes.

limited genetic variability within *Austrolestes* and *Ischnura*. The same was true for *Xanthocnemis zealandica* (n = 151), which had very limited levels of allelic variation, low percentages of polymorphic loci (≤9% at 12 of 13 sites) and low heterozygosity (average = 2%).

Heterozygosity levels were lower than those previously reported for other New Zealand aquatic insects (e.g., 5.7% for *Archicauliodes diversus*; Hogg et al. 2002), but comparable to those reported for the North American mayfly *Dolania americana* (average = 3%; Sweeney & Funk 1991).

Allelic variability was comparable to levels previously reported for New Zealand freshwater insects (Smith & Collier 2001; Hogg et al. 2002), but lower than the 3–4 alleles per locus reported for North American taxa (e.g., Robinson et al. 1992; Plague & McArthur 1998). Limited allelic variability may be a prevalent feature among New Zealand freshwater insects, and is possibly the result of the insular nature of the New Zealand land mass.

Measures of genetic differentiation among the sampled habitats were also low. Wright's (1978) F_{ST} values were similar to those reported for New Zealand mayflies, but lower than the caddisfly *Orthopsyche fimbriata* and megalopteran *Archicauliodes diversus* (Smith & Collier 2001; Hogg et al. 2002, respectively). This suggests that levels of dispersal and hence gene flow are adequate to maintain an almost homogenous population structure for damselflies in New Zealand. The presence of both *Xanthocnemis* and *Austrolestes* on Chatham Islands also suggests that they have moderate dispersal capabilities, as these islands became available for colonisation only in the last 3–5 million years (Nelson et al. 1999). However, the limited distribution of some *Xanthocnemis* species (e.g., *X. sobrinia*, *X. tuanuii*; Rowe 2000) suggests that dispersal pathways or other factors (e.g., competition, habitat requirements) may have some influence on distribution.

In summary, we found genetic techniques to be useful in distinguishing between species, including

the closely related *X. zealandica* and *X. tuanuii*, and may further prove useful in identifying morphologically similar larvae (e.g., *Xanthocnemis* spp.). We also found limited genetic variability and differentiation among locations for species of New Zealand damselflies—as expected for relatively long-lived taxa with moderate dispersal capabilities. Future work targeting other less common odonate species (e.g., anisopterans) is needed to accurately document the biodiversity of New Zealand.

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